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DOI: <https://doi.org/10.1093/hmg/2.12.1991>

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ZORA URL: <https://doi.org/10.5167/uzh-154629>

Journal Article

Published Version

Originally published at:

Bulting, Karin; Dlttrich, Bärbel; Groß, Stephanle; Greger, Valerle; Lalande, Marc; Robinson, Wendy; Mutirangura, Apiwat; Ledbetter, David; Horsthemke, Bernhard (1993). Molecular definition of the Prader — Willi syndrome chromosome region and orientation of the SNRPN gene. *Human Molecular Genetics*, 2(12):1991-1994.

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Molecular definition of the Prader – Willi syndrome chromosome region and orientation of the SNRPN gene

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Received August 31, 1993; Revised and Accepted October 14, 1993

The Prader – Willi syndrome and the Angelman syndrome are caused by the loss of function of distinct but closely linked genes on human chromosome 15. Based on a yeast artificial chromosome restriction map and two key patients we have determined that the shortest region of deletion overlap in the Prader – Willi syndrome comprises 320 kb. The region includes the anonymous DNA marker PW71 (D15S63) and the gene for the small nuclear ribonucleoprotein N (SNRPN). The SNRPN gene maps 130 kb distal to PW71 and is transcribed from centromere to telomere.

INTRODUCTION

The Prader – Willi syndrome (PWS) and the Angelman syndrome (AS) are distinct neurogenetic disorders, which are caused by the loss of function of closely linked genes on chromosome 15. Paternal deletions and maternal disomy in PWS suggest that the PWS gene(s) are transcribed from the paternal chromosome only. Likewise, maternal deletions and paternal disomy in AS suggest that the AS gene(s) are transcribed from the maternal chromosome only (for review see reference 1).

About 60% of patients with PWS and AS have a deletion that includes all the loci from ML34 (D15S9/ZNF127) to IR10 (D15S12/P) (Fig. 1). A complete YAC contig spanning this region has recently been developed (2). Robinson et al. (3) have described a unique PWS patient (PW93), who is deleted for a subset of these loci only. The proximal deletion breakpoint in this patient is between 189-1 (D15S13) and PW71 (D15S63) and currently defines the proximal boundary of the PWS region. Saitoh et al. (4) have described a Japanese family in which a deletion encompassing 3-21 (D15S10), LS6-1 (D15S113) and GABRB3 causes AS when transmitted through the female germline, but is without phenotype when transmitted through the male germline. Thus, the proximal deletion breakpoint in this family defines the distal boundary of the PWS region. A genomic DNA fragment spanning this boundary has recently been cloned (B1.5, D15S174) (5).

The PWS region includes the anonymous DNA marker PW71, which shows parent-of-origin specific DNA methylation (6), and the gene for the small nuclear ribonucleoprotein protein N (SNRPN), which has been proposed as a candidate gene for PWS (7). Based on yeast artificial chromosome clones (8) we have determined the size of this region and constructed a detailed physical map.

RESULTS

Five yeast artificial chromosome clones (YACs) were analyzed. YAC B58C7 had been identified with probe PW71. The left end of this YAC contains non-chromosome 15 DNA. The right end of this YAC was used to isolate YACs 11H11, 309G7, 326F6, and 457B4 (8). Total yeast DNA was digested with the rare cutter enzymes *SacII*, *SaII* and *NotI* and separated by pulsed field gel electrophoresis. Southern blots were hybridized with total human DNA to detect all restriction fragments, with appropriate pBR322 fragments to detect YAC end fragments (9) and with several unique probes from this region (Table 1).

The restriction enzyme analysis revealed 3 *SacII* sites, 2 *SaII* sites and 2 *NotI* sites. As shown in Fig. 2, YAC 326F6 spans 189-1 and PW71. These loci are separated by a 180 kb *SacII* fragment. YACs 11H11 and 309G7 span PW71 and SNRPN. YAC B58C7 is positive for PW71 only and YAC 457B4 is positive for SNRPN only. A subclone from YAC B58C7, which maps 3 kb telomeric to PW71 and contains a long terminal repeat (LTR) (see accompanying paper by Ditttrich et al., (10)), detects an abnormal *HindIII* fragment in YAC 457B4 (not shown). This indicates that the right end of YAC 457B4 maps very close to PW71.

Another subclone from B58C7 (p71.19.11, (10)) and an inter-Alu PCR clone (p9.2) were tested. Both probes identify a 180 kb *SacII* fragment in YAC 326F6 and a 95 kb *SacII* fragment in YAC B58C7. This indicates that 9.2 and 71.19.11 map centromeric to the *SacII* site in YAC B58C7. PW71 identifies a 30 kb *SacII* fragment and thus maps telomeric to this site.

The proximal deletion breakpoint in patient PW93 had been mapped to the region between 189-1 and PW71 (3). For localizing this breakpoint more precisely we hybridized 9.2 and 71.19.11 to *HindIII* digested DNA from this patient. MetD was used as

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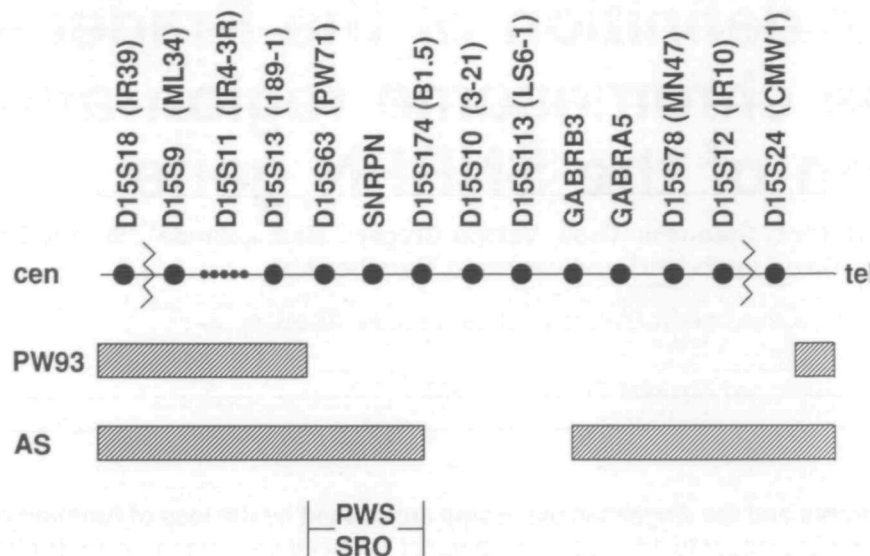


Figure 1. Locus order in 15q11-13. The wavy lines indicate the common deletion breakpoints in AS and PWS. The chromosome regions which are intact in the PWS patient PW93 (2) and the Japanese AS family (3) are indicated by hatched bars. The distance between the loci are not drawn to scale. SRO, shortest region of deletion overlap.

an internal hybridization standard. Densitometric analysis revealed two copies of 9.2 (not shown). 71.19.11, which maps between 9.2 and PW71, detects *Hind*III, *Bcl*I and *Bgl*II deletion junction fragments in the patient (Fig.3). The detection of an abnormal *Hind*III fragment by 71.19.11, which is a 600 bp *Sau*3A-*Hind*III (cen→tel) fragment (10), indicates that 71.19.11 spans the proximal deletion breakpoint in PW93.

Probe B1.5 (D15S174), which defines the distal boundary of the PWS region (5), maps to a 60 kb *Sal*I fragment from the left (telomeric) end of YAC 457B4. Since the corresponding fragment from YAC 309G7 is 50 kb and negative for B1.5, this marker maps within 10 kb from the genomic end of YAC 457B4. Based on this result and the localization of the PW93 deletion breakpoint we can estimate that the PWS region is approximately 320 kb.

Analysis of the YAC clones with *Not*I revealed two sites: one 170 kb centromeric to PW71 and one 130 kb telomeric to PW71. The latter site most probably corresponds to the *Not*I site present at the 5' end of the SNRPN cDNA (11). Using a brain cDNA library and appropriate PCR primers, we amplified the SNRPN cDNA sequence 3' to the *Not*I site. This fragment hybridizes to the telomeric *Not*I fragments of YACs 11H11, 309G7 and 457B4. This indicates that the SNRPN gene maps approximately 130 kb telomeric to PW71 and is transcribed from centromere to telomere.

DISCUSSION

Based on a YAC restriction map and two key patients we have determined that the shortest region of deletion overlap in PWS currently comprises 320 kb. The region includes the SNRPN gene, which in mice is subject to genomic imprinting (12). This makes SNRPN a good candidate gene for at least some of the features of PWS. By anchoring the gene to a *Not*I site, which may be part of a CpG island, we have determined that the direction of transcription is from centromere to telomere.

Table 1. YAC restriction fragments

YAC clone	size	Enzyme <i>Sal</i> I	<i>Sac</i> II	<i>Not</i> I
326F6	300 kb	250 kb R, 189-1 50 kb L, PW71	180 kb R, 9.2 70 kb R, 189-1 50 kb L, PW71	220 kb 80 kb
11H11	420 kb	265 kb PW71, SNRPN 135 kb R 20 kb L	130 kb PW71 155 kb R 135 kb L, SNRPN	155 kb SNRPN 265 kb
309G7	440 kb	265 kb PW71, SNRPN 125 kb L 50 kb R	130 kb PW71 185 kb R, SNRPN 125 kb L	185 kb SNRPN 255 kb
B58C7	125 kb	95 kb L 30 kb R, PW71	95 kb L, 9.2 30 kb R, PW71	125 kb
457B4	315 kb	255 kb R, SNRPN 60 kb L, B1.5	195 kb L, SNRPN, B1.5 120 kb R	195 kb SNRPN 120 kb

Restriction fragments were detected by hybridization with total human DNA, unique probes (indicated by superscripts) and pBR322 fragments for the right (R) and left (L) YAC ends. *Not*I digests were hybridized with total human DNA and SNRPN only. Probes 9.2 and 71.19.11 were used on *Sac*II digested DNA of YACs 326F6 and B58C7 only.

The PWS SRO is large enough to contain many more genes beside SNRPN. Apart from a *Sal*I and *Sac*II site close to PW71, however, there is no indication for another CpG island in this region. It is possible that most of the genes in this region lack such islands. YAC 457B4, which spans most of the PWS SRO, will be a useful tool to search for additional genes. A good candidate region for gene sequences is the PW71 locus, which displays parent-of-origin specific DNA methylation (5). It contains a 'solo' LTR, which may promote the expression of a nearby gene (10).

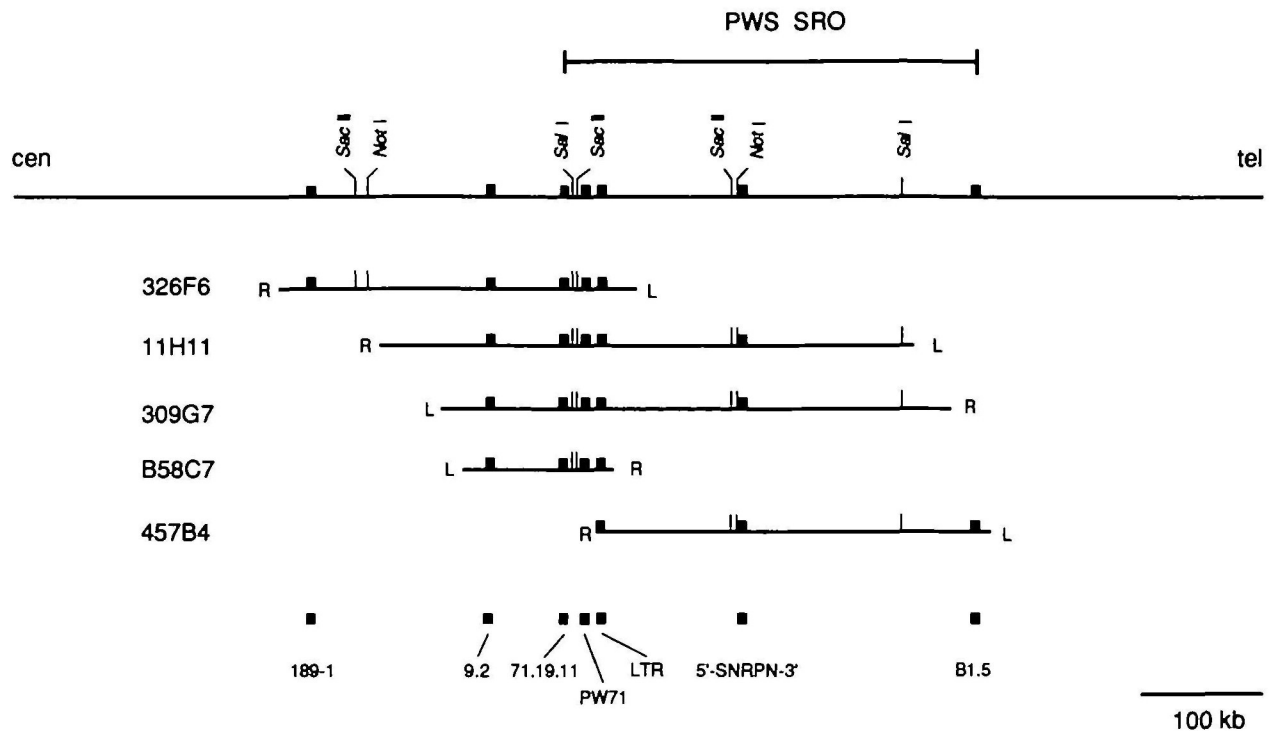


Figure 2. Restriction map of the PWS SRO. The restriction map was constructed from the data shown in table 1. The exact position of 189-1 and 9.2 within the indicated restriction fragments are unknown.

The fact that no PWS patient with a SNRPN point mutation has been described to date may indicate that the loss of function of the SNRPN gene is not sufficient for typical PWS. It is possible that the development of the full PWS phenotype requires the loss of additional genes. It should be noted that these genes may not necessarily map to the shortest region of deletion overlap. Glenn et al. (13) and Reis et al. (Reis, A., Dittrich, B., Greger, V., Buiting, K., Lalande, M., Gillissen-Kaesbach, G., Anvret, M. and Horsthemke, B., in preparation) have described AS and PWS patients who appear to have normal chromosomes of biparental origin but abnormal methylation patterns at the ZNF127/D15S9 and the D15S63 loci. Furthermore, these authors have obtained evidence for long range position effects of chromosomal rearrangements on the methylation pattern of these loci. Thus, it may ultimately be necessary to generate a complete transcription map of 15q11–13 and to study the expression of these genes.

MATERIAL AND METHODS

Pulsed field gel electrophoresis

High molecular weight yeast DNA in agarose plugs was prepared as described by Carle and Olson (14). After digestion with the appropriate enzymes, DNA fragments were separated by transverse alternating field electrophoresis (TAFE, Beckman Instruments) in 10 mM Tris/HCl/0.5 mM EDTA (free acid)/4.4 mM acetic acid, pH 8.2. Running conditions were 1 hr with 4 sec pulses at 170 mA followed by 20 hr with 25 sec pulses or 18 hr with 10 sec pulses at 150 mA. *Saccharomyces cerevisiae* chromosomes (strain AB1380), lambda-concatamers from lambda Sam 7 and lambda DNA digested with *HindIII* were used as size markers. Southern blots were prepared and hybridized as described (15). The final wash was usually at 65°C in 300 mM sodium chloride/30 mM sodium citrate(2×SSC) containing 0.1% SDS.

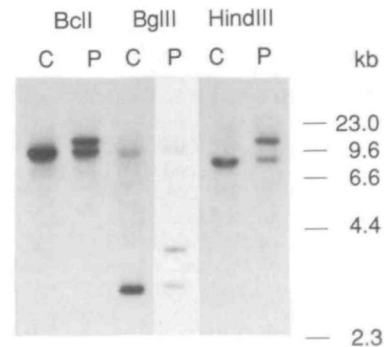


Figure 3. Proximal deletion breakpoint in patient PW93. DNA samples from PW93 (P) and a normal control (C) were digested with *BclI*, *BglII* and *HindIII* and probed with 71.19.11. The probe detects deletion junction fragments in the patient. The size markers were generated by *HindIII* digestion of lambda DNA.

DNA probes

PW71, 189-1 and B1.5 have been described before (16,17,5). BR322R and BR322L are pBR322 fragments that detect the right and left ends of the YAC vector, respectively (9). MetD, a probe from chromosome 7, was used as an internal standard. 71.19.11 is a genomic subclone from YAC B58C7 isolated by Dittrich et al. (10). 9.2 is an inter-alu-PCR product amplified with the alu primer PDJ34 from YAC B58C7. Primer sequence and amplification was performed as described by Nelson et al. (18). A probe for SNRPN was isolated from a brain cDNA library by PCR. Based on the sequence of the human SNRPN cDNA (11), a set of PCR primers were synthesized (SmN1, 5' AGTGTGAGT-TGTACCCGAGG 3'; SmN2 5' CAGGGGAAAAAGTGACTGAG 3'). The primers were used to amplify a 1 kb cDNA fragment from a fetal brain cDNA library (Stratagene). PCR was carried out in a GenAmp PCR system 9600 (Perkin-Elmer/Cetus) in a total volume of 100 µl containing 2×10^7 pfu of the cDNA

library, 50 mM KCl, 10 mM Tris-HCl (pH8.0), 1.5 mM MgCl₂, 0.01% gelatin, 200 mM each dNTP and 2.5 units Taq DNA polymerase. The following conditions were used: 35 cycles of denaturation (95°C for 15 sec), annealing (58°C for 30 sec) and extension (72°C for 30 sec).

ACKNOWLEDGEMENT

We would like to thank B. Brandt for expert technical assistance and Prof. E. Passarge for continuous support. Parts of this work were supported by the Deutsche Forschungsgemeinschaft.

REFERENCES

1. Nicholls, R.D. (1993) *Current Opinions in Genetics and Development* 3, 445-456.
2. Mutirangura, A., Jayakumar, A., Sutcliffe, J.S., Nakao, M., McKinney, M.J., Buiting, K., Horsthemke, B., Beaudet, A.L., Chinault, A.C. and Ledbetter, D.H. (1993) *Genomics*. In Press.
3. Robinson, W.P., Bottani, A., Yagang, X., Balakrishnan, J., Binkert, F., Mächler, M., Prader, A. and Schinzel, A. (1991) *Am. J. Hum. Genet.* 49, 1219-1234.
4. Saitoh, S., Kubota, T., Ohta, T., Jinno, Y., Niikawa, N., Sugimoto, T., Wagstaff, J. and Lalande, M. (1992) *Lancet* 339, 366-367.
5. Greger, V., Woolf, E., and Lalande M. (1993) *Hum. Molec. Genet.* 2, 921-924.
6. Dittrich B., Robinson, W.P., Knoblauch, H., Buiting, K., Schmidt, K., Gillissen-Kaesbach, G. and Horsthemke B. (1992) *Hum. Genet.* 90, 313-315.
7. Özcelik, T., Leff, St. Robinson, W., Donlon, T., Lalande M., Sanjines E., Schinzel, A. and Francke, U. (1992) *Nature Genet.* 2, 265-269.
8. Kuwano, A., Mutirangura, A., Dittrich, B., Buiting, K., Horsthemke, B., Saitoh, S., Niikawa, N., Ledbetter, S.A., Greenberg, F., Chinault, A.C. and Ledbetter, D.H. (1992) *Hum. Molec. Genet.* 1, 417-425.
9. Burke, D.T., Carle, G.F. and Olson M.V. (1987) *Science* 236, 806-812.
10. Dittrich, B., Buiting, K., Groß S. and Horsthemke B., in preparation.
11. Schmauss, C., Mc Allister, G., Ohosone, Y., Hardin, J.A. and Lerner, M.R. (1989) *Nucleic Acids Res.* 17, 1733-1743.
12. Leff St.E., Brannan, C.I., Reed, M.L., Özcelik, T., Francke, U., Copeland, N.G. and Jenkins N.A. (1992) *Nature Genet.* 2, 259-264.
13. Glenn, C.C., Nicholls R.D., Robinson W.P., Saitoh, S., Niikawa, N., Schinzel, A., Horsthemke B. and Driscoll D.J. (1993), *Hum. Molec. Genet.* 2, 1377-1382.
14. Carle, G.F. and Olson, M.V. (1984) *Nucleic Acids Res.* 12, 5647-5664.
15. Horsthemke, B., Greger, V., Barnert, H.J., Höpping, W. and Passarge, E. (1987) *Hum. Genet.* 76, 257-261.
16. Buiting, K., Neumann, M., Lüdecke, H.-J., Senger, G., Claussen, U., Antich, J., Passarge, E. and Horsthemke, B. (1990) *Genomics* 6, 521-527.
17. Donlon, T.A., Lalande, M., Wyman, A., Bruns, G. and Latt, S. A. (1986) *Proc. Natl. Acad. Sci. USA* 83, 4408-44115.
18. Nelson, D.L., Ledbetter, S.A., Corbo, L., Victoria, M.F., Ramirez-Solis, R., Webster, T.D. and Ledbetter, D.H (1989) *Proc. Natl. Acad. Sci. USA* 86, 6686-6690.